EVOLUTION OF CATALYTIC RNA IN THE LABORATORY

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It is believed that an RNA-based genetic system, usually referred to as the "RNA world", preceded the DNA and protein-based genetic system that has existed on Earth for the past 3.5 billion years. Questions concerning how the RNA world arose and the degree of complexity it attained can be addressed through laboratory experiments in prebiotic chemistry and RNA biochemistry. In the realm of prebiotic chemistry, one seeks to explain how the chemical components of RNA arose and assembled to form polynucleotides in the presence of a complex mixture of closely-related compounds. Ribose, for example, would have been accompanied by many other sugars, and is more reactive and degrades more rapidly than these other sugars. Taking advantage of its greater reactivity, we found that ribose reacts especially rapidly with cyanamide to form a stable bicyclic adduct. This product crystallizes readily in aqueous solution, whereas other sugar-cyanamides present in the same mixture do not. The ribose-cyanamide crystals derived from racemic ribose contain a mosaic of pure-D and pure-L domains. Ribose-cyanamide in turn reacts with cyanoacetylene to form cytosine alpha-nucleoside in nearly quantitative yield. This reaction takes place either with ribose-cyanamide in solution or in crystals, employing an aqueous solution of 50 mM cyanoacetylene over a broad range of conditions. The much greater efficiency of this reaction compared to previous reports is due to the finding that higher concentrations of cyanoacetylene (as employed previously) are deleterious because they promote a side reaction of cyanoacetylene with cytosine nucleoside. Central to the operation of the RNA world is the ability of RNA to catalyze the replication of RNA, thereby enabling RNA-based evolution. Through methods of test-tube evolution, we have developed RNA enzymes that catalyze the template-directed joining of RNA. We evolved one such enzyme that contains only three of the four subunits of RNA (A, U, and G, but lacking C). It subsequently was evolved to produce a more reactive variant that contains all four subunits, as well as a less reactive variant that contains only two subunits (2-amino-A and U). Most recently, the four-subunit variant was evolved to contain deoxynucleotides rather than ribonucleotides. This demonstrates how catalytic function can be retained during the conversion between an RNA and RNA-like (in this case DNA) molecule. The four-subunit ligase described above was used to construct a self-replicating ribozyme that catalyzes the ligation of two RNA substrates to form additional copies of itself. This in turn was used to construct a pair of cross-replicating ribozymes that catalyze each other's synthesis from a total of four component substrates. The rate of formation of the two ribozymes increases progressively over the course of the reaction, consistent with the overall autocatalytic behavior of the system.